Supplemental material for

Substrate specificity of an elongation-specific peptidoglycan endopeptidase and its implications for cell wall architecture and growth of *Vibrio cholerae*

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Running title: Control of cell elongation in *Vibrio cholerae*

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Supplementary Figure legends

Fig. S1. Predicted domain structures of the three *V. cholerae* proteins predicted to contain OapA and M23 domains

Domain structures were predicted using BLAST (NCBI). Signal sequences were predicted using SignalP V 4.1 (http://www.cbs.dtu.dk/services/SignalP/).

Fig. S2. Alignment of ShyA, ShyB and ShyC protein sequences. Alignments were done using the Praline web server (Simossis & Heringa, 2005). The predicted transmembrane segment of ShyC is framed in white, the predicted OapA domains in black and the signature M23 domain catalytic site in blue.

Fig. S3. ShyA depletion in a Δ*shyC* background on agarose pads.

(A) Cells were treated as described in the legend for Fig. 2 C. The arrowhead points to protruding membrane vesicles. ShyA + is TD541 (Δ*shyA* Δ*shyC* P_{IPTG}:*shyA*) on glucose/IPTG; ShyA – is TD541 on glucose alone. Frames are 5 min (ShyA-) or 2 min (ShyA +) apart. (B) Cell were treated as described above. Wild type was plated on glucose. (C) Cells were grown as described in the legend of Fig. 2C. Cell volume was measured on cells plated on agarose pads containing glucose (ShyA -) or glucose + IPTG (ShyA +) using MicrobeTracker and Matlab.

Fig. S4. Western blot analysis of mCherry fusions.

Cells expressing the respective mCherry fusions were grown under the same conditions as those used for fluorescent imaging. Cells were then lysed and blotted with anti-mCherry antibody. pHL100 = empty vector control.

Fig. S5. HPLC-quantification of PG released over time from sacculi by digestion with ShyA. For comparison, PG released by full solubilization of sacculi with muramidase is also shown. AU=arbitrary units

Fig. S6. Determination of the composition of the PG-chains solubilized by ShyA.

(A) HPLC chromatogram of ShyA-digested sacculi. Solubilized muropeptides (labeled with numbers 1-10) were not reduced prior to injection. (B) HPLC analysis of peak 2 after digestion with muramidase shows generation of 2 new peaks (2a and 2b). (C) Measured and theoretical masses of PG peaks 2, 2a, 2b (same as 1) and 3 from chromatograms A and B. (D) Structure representations of the muropeptides identified in (C). (E) HPLC chromatograms and (F) M4/M4N ratio of the PG peaks (numbers 4-10 from panel (A)) solubilized by ShyA. Samples were subjected to muramidase digestion prior injection in the HPLC. R.A: Relative abundance and SD: standard deviation. Data correspond to mean values and SD of three independent experiments.

Fig. S7. Representative HPLC chromatograms

(A) Representative HPLC chromatograms of muramidase-digested PG samples of *V. cholerae* wild type, the **Δ***shyA* mutant and the insoluble fraction of PG partially (as described for Fig. 5C) digested with ShyA (PG + ShyA). (B) Quantification of the relative abundance of monomers, dimer and trimers as well as crosslinkage and average chain length. Relative molar abundance of muropeptides was calculated from the areas of the corresponding peaks as described previously (Glauner et al., 1988).

Table S1. Oligos used in this study

Bases in bold are homologous overhangs.

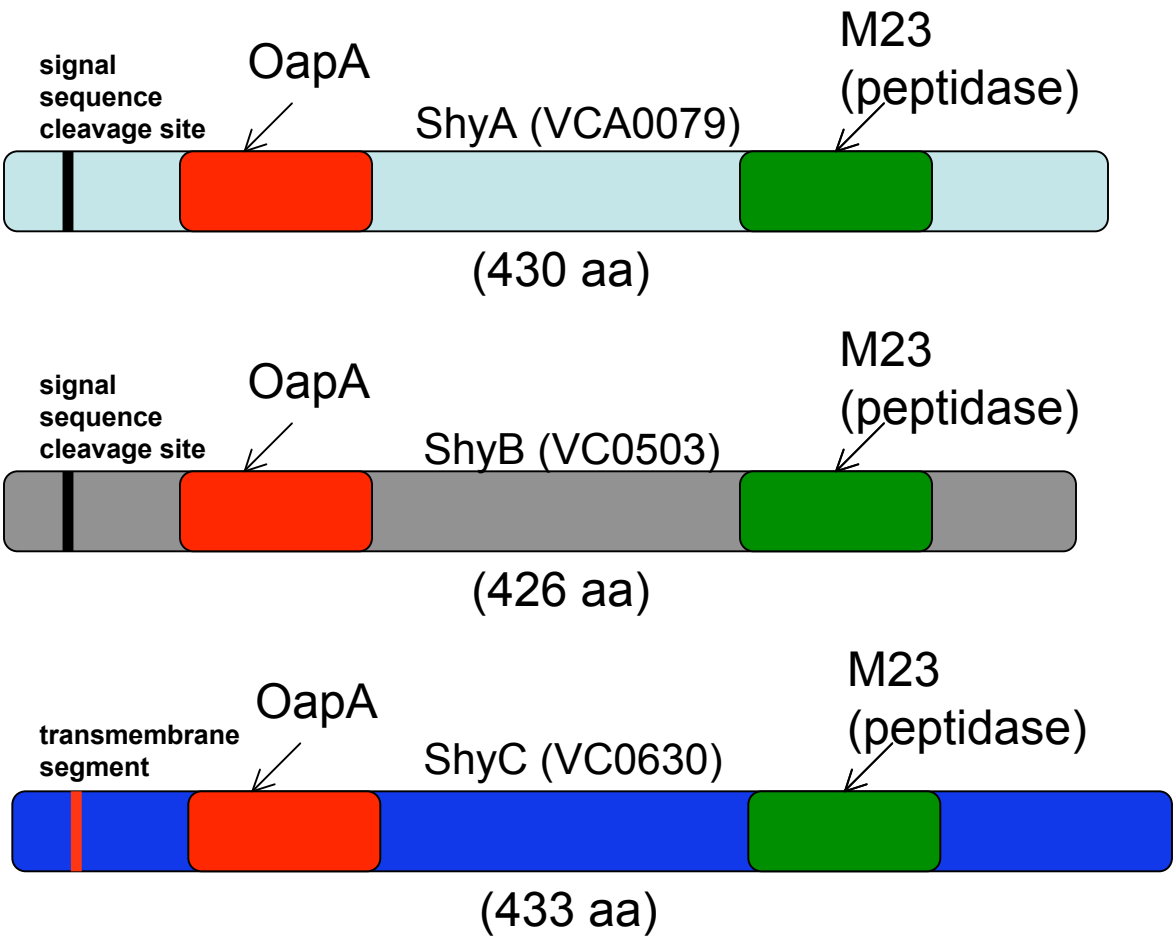

signal
sequence
cleavage site
OapA
ShyA (VCA0079)
M23
(peptidase)
(430 aa)
signal
sequence
cleavage site
OapA
ShyB (VC0503)
M23
(peptidase)
(426 aa)
transmembrane
segment
OapA
ShyC (VC0630)
M23
(peptidase)
(433 aa)

Fig.S1

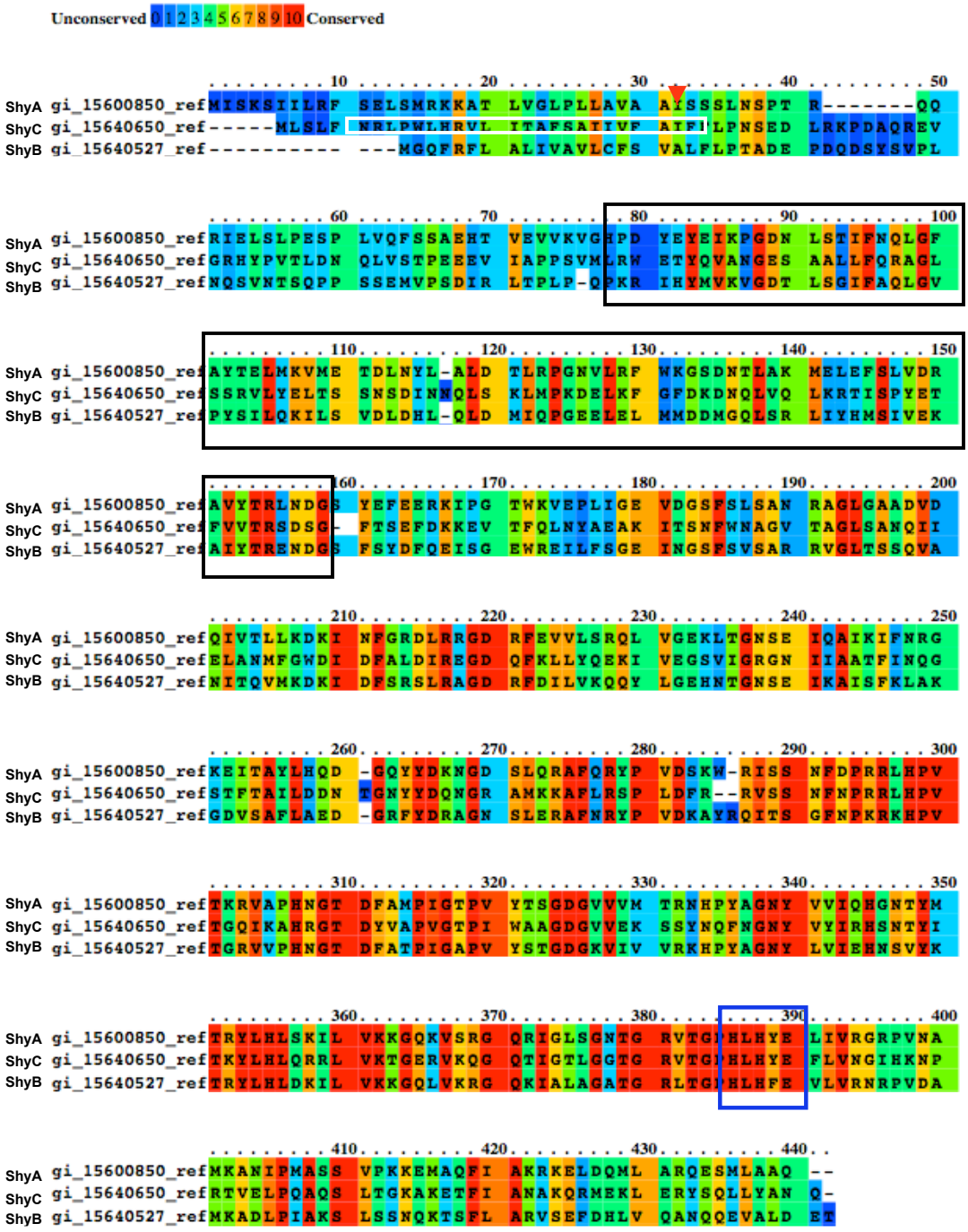
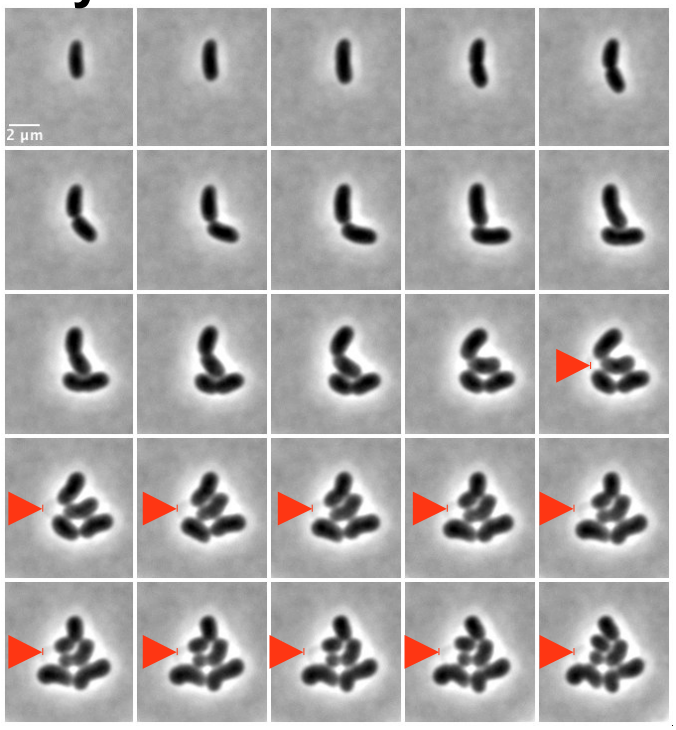
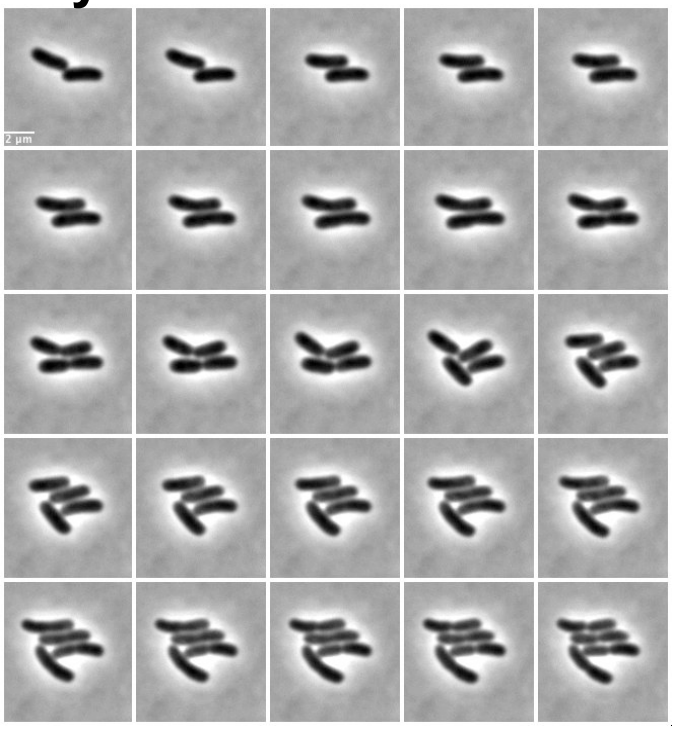

Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved
ShyA gi_15600850_ref
ShyC gi_15640650_ref
ShyB gi_15640527_ref

Fig.S2

A ShyA -



ShyA +



B

strain	width(µm) +/- SD
Wt (n=1964)	0.6 +/- 0.05
ShyA + (n=1122)	0.7 +/- 0.06
ShyA - (n=1977)	0.94 +/- 0.1

C

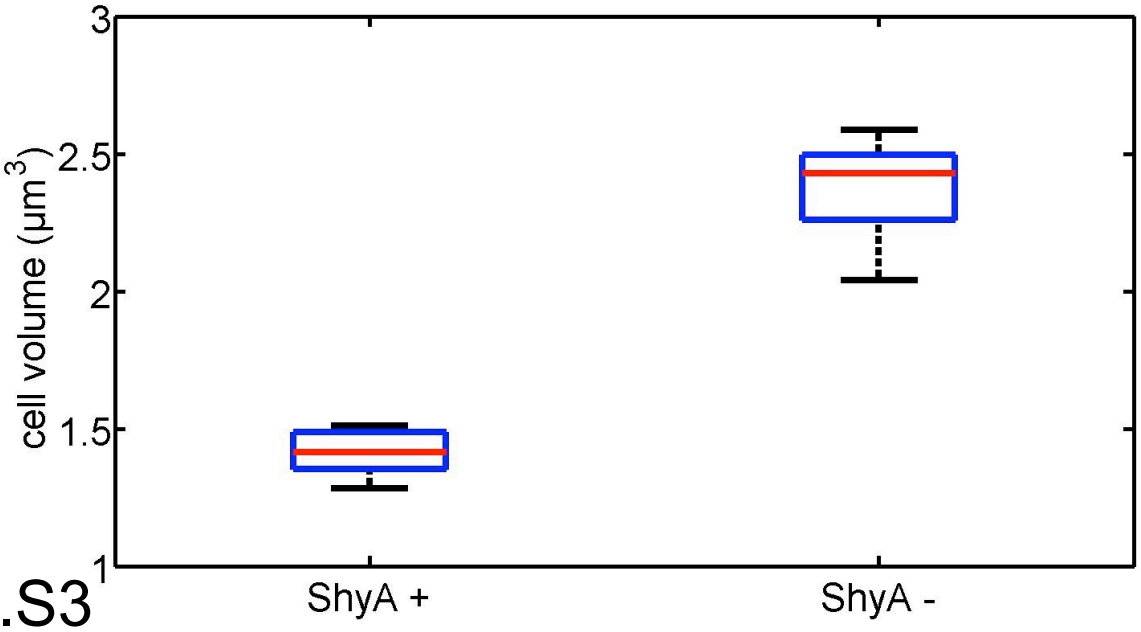


Fig.S3

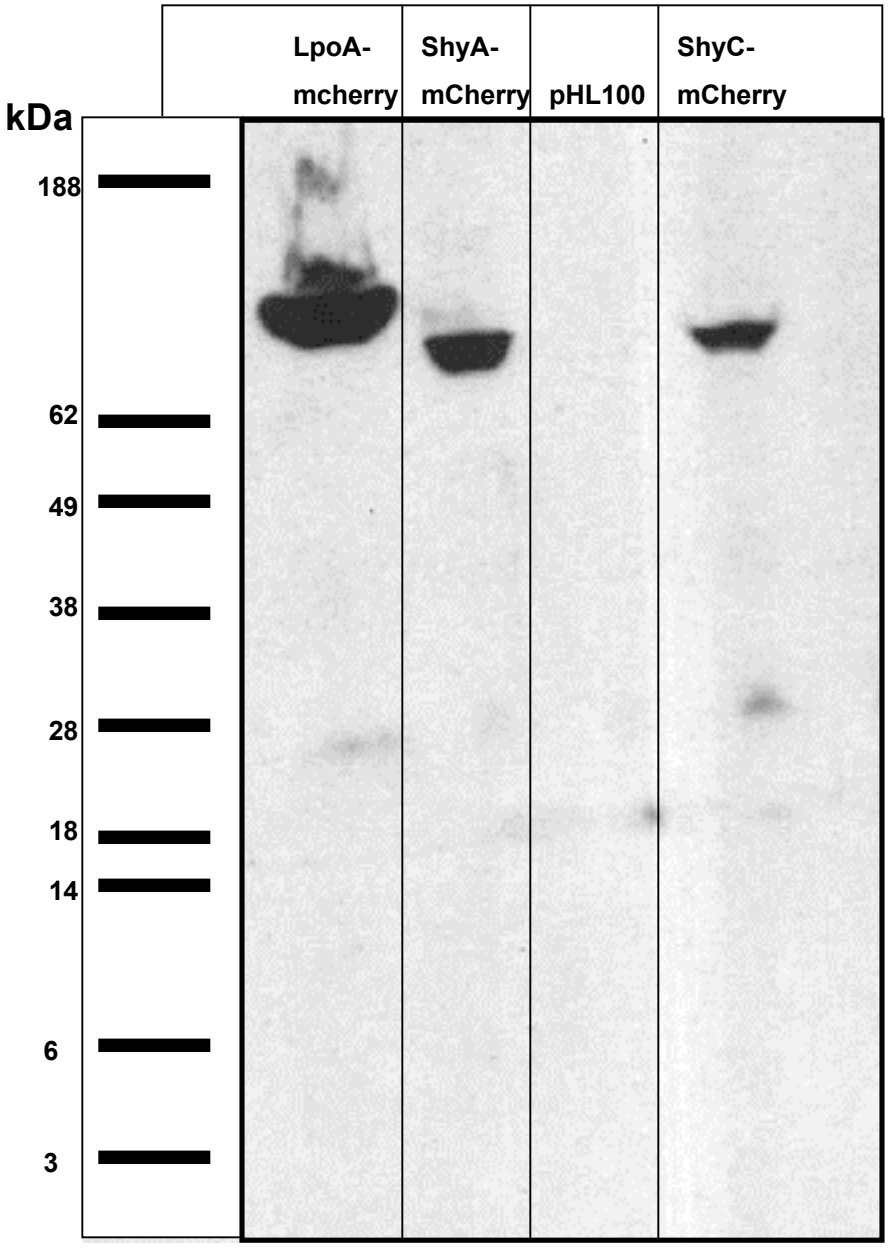

LpoA-
mcherry
ShyA-
mCherry
pHL100
ShyC-
mCherry
kDa
188
62
49
38
28
18
14
6
3

Fig.S4

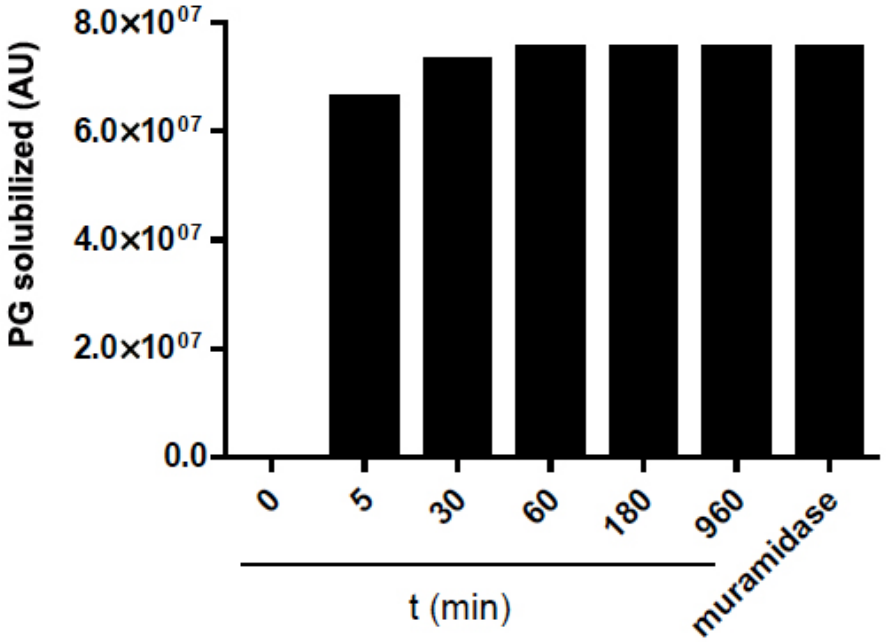
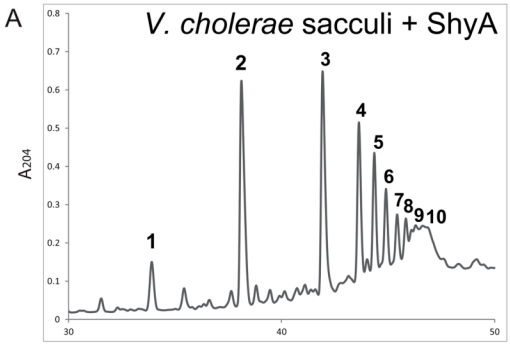
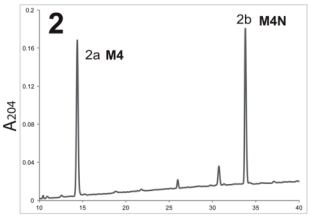

PG solubilized (AU)
8.0×10^07
6.0×10^07
4.0×10^07
2.0×10^07
0.0
0
5
30
60
180
960
muramidase
t (min)

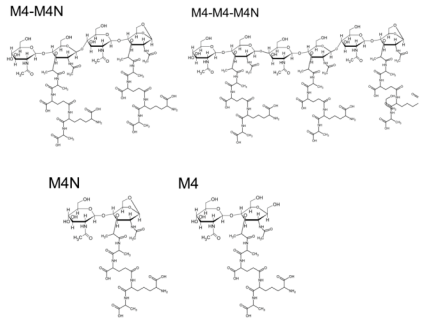
Fig.S5

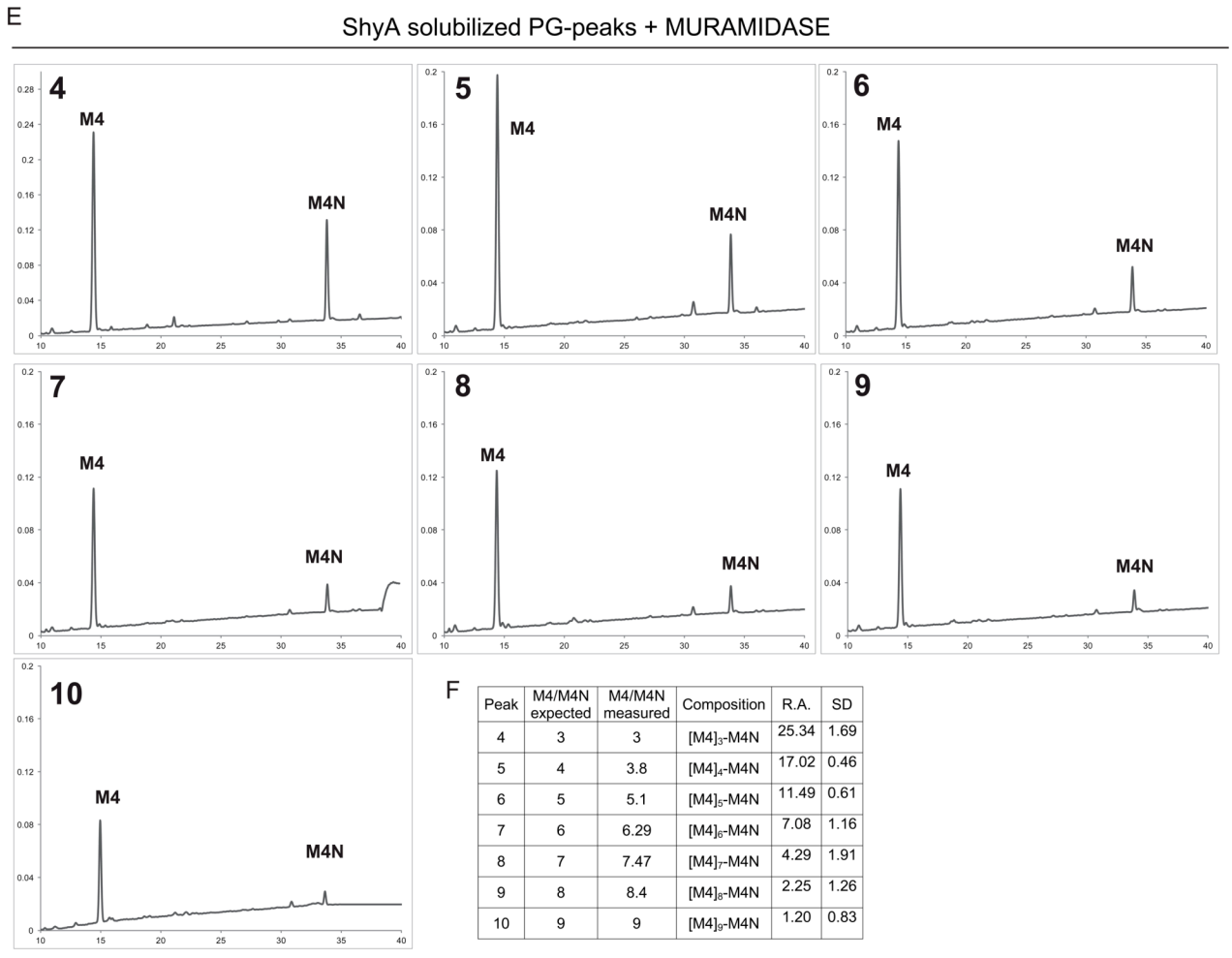




C

Peak	Expected	Measured [M+H]+	Difference (exp-mes)	Composition
2	1843.76	1843.57	0.19	M4-M4N
3	2765.14	2765.18	0.04	M4-M4-M4N
2a	940.39	940.30	0.09	M4
2b	922.41	922.22	0.19	M4N



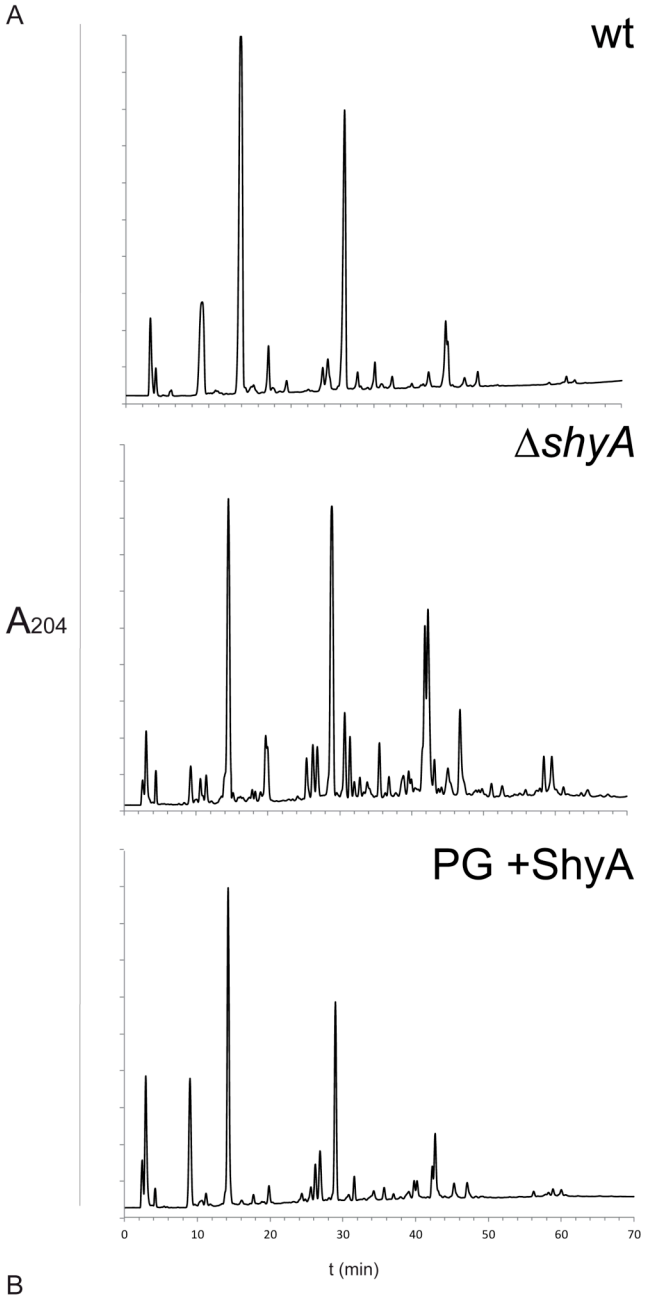


F

Peak	M4/M4N expected	M4/M4N measured	Composition	R.A.	SD
4	3	3	$[M4]_3$-M4N	25.34	1.69
5	4	3.8	$[M4]_4$-M4N	17.02	0.46
6	5	5.1	$[M4]_5$-M4N	11.49	0.61
7	6	6.29	$[M4]_6$-M4N	7.08	1.16
8	7	7.47	$[M4]_7$-M4N	4.29	1.91
9	8	8.4	$[M4]_8$-M4N	2.25	1.26
10	9	9	$[M4]_9$-M4N	1.20	0.83

Fig.S6

A



B

	wt	Δ*shyA*	PG+ShyA
monomers	63.82	55.09	76.16
dimers	34.40	40.97	22.80
trimers	1.79	3.94	1.04
cross-linkage	**37.97**	**48.85**	**24.88**
average length	**8.08**	**3.98**	**13.20**

Fig.S7

Table S1

oligo	description	sequence
TDP498	VCA0079uphomfwpCVD	**accgcatgcgatatcgagctctccc**AGGGCGGCTTCAGTTGACT
TDP499	VCA0079uphomrevLINK	**TTAtcaTGCGGCCGCACTCGAGTAATGATAA**CAGTTTACCTGTGGAAAAAAGTCAAAACTCG
TDP500	VCA0079dwnhomfwLINK	**TTATCATTACTCGAGTGCGGCCGCAtgaTAA**ATCGAGTATGAGTACAAAGCCCCG
TDP501	VCA0079dwnhomrevpCVD	**TTGTGAGCGGATAACAATTTGTGGAATTCCC**CCATTGGAATTCATTGAAGCAGCAG
TDP685	VC0503fwhompDS	**aggtatatgtgatgggttaaaaaggatcgatcct**CTTCCCGATATCTTGCCCTTCAATT
TDP686	VC0503revhom	**TTAtcaTGCGGCCGCACTCGAGTAATGATAA**AGAAATCTAAATTGACCCATGAGAACTAAGCA
TDP687	VC0503dwnhomfw	**TTATCATTACTCGAGTGCGGCCGCAtgaTAA**AGAGGTTGCTCTCGACGAAACTTAA
TDP688	VC0503dwnhomrev	**ccgggagagctcgatatcgcatgcggtacctctag**AACACTCTACTCCGATACGCCG
TDP689	VC0630uphomfw	**aggtatatgtgatgggttaaaaaggatcgatcct**AGGAAGGGTACAACCTAGCGC
TDP690	VC0630uphomrev	**TTAtcaTGCGGCCGCACTCGAGTAATGATAA**TCATCTCGTGGCGGACAAAAA
TDP691	VC0630dwnhomfw	**TTATCATTACTCGAGTGCGGCCGCAtgaTAA**TTGAAAAGAGAAAGCATGTTCAGTTCAAATTC
TDP692	VC0630dwnhomRev	**ccgggagagctcgatatcgcatgcggtacctctag**CACACCAATATAGTTATGCGCTGATTTGG
TDP513	VCA0079revHISpet	**agccggatctcagtggtggtggtggtggtgctcga**TTAgtggtgatggtgatgatgTTGCGCTGCTAGCATGC
TDP519	VCA0079truncfwPET	**aaataattttgtttaactttaagaaggagatatac**ATGCTAAACAGTCCCACGCGGCAA

TDP595	shyAH375Afw	CGTGTGACCGGTCCTgcTCTGCACTATGAG
TDP596	shyAH375Arev	CTCATAGTGCAGAgcAGGACCGGTCACACG
TDP529	VCA0079fwpHL	**accatggaattcgagctcggtaccc**GACTTTTTTCCACAGGTAAACTGGTGA
TDP530	VCA0079revpHL	**tgcaggtcgactctagaggatcccc**CGGGGCTTTGTACTCATACTCGAT
TDP518	0079revmCherry	**TGTTATCCTCCTCGCCCTTGCTCACGGTCGCCACCGGCGG**TTGCGCTGCTAGCATGCTTT
TDP238	mCherryfwlpoA	GTGAGCAAGGGCGAGGAGG
TDP239	mCherryrevpHL	**tgcaggtcgactctagaggatcccc**TTACTTGTACAGCTCGTCCATGC
TDP674	pHLfwpJL	**gtgatgattggtacCAGATCTTAATTAAGG**GTACCGCcgtcatcaccgaaacgcgc
TDP675	pHLrevpJL	**cggggattgGTACCGCGGCCGCTCTAGAGG**tgcgttctgatttaatctgtatcaggct
TDP753	0630fwpHL	accatggaattcgagctcggtacccTGAATTTGAACTGAACATGCTTTCTCTTTT
TDP723	0630revmCherry	GATGGCCATGTTATCCTCCTCGCCCTTGCTCACgagctcgaggatgtcGGGCAGAAAAAAAATGGCGAAGACA